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(71) Applicant (for all designated States except US): GROW GREEN PTY. LTD. [AU/AU]; 47 Wyuna Court, Hemmant, QLD 4174 (AU).

(72) Inventor; and

(75) Inventor/Applicant (for US only): ANVARI, Frank [AU/AU]; 47 Wyuna Court, Hemmant, QLD 4174 (AU).

(74) Agent: PIZZEYS PATENT AND TRADE MARK ATTOR-NEYS; GPO Box 1374, Brisbane, QLD 4001 (AU).

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(54) Title: PROTEIN FEEDSTOCK, COMPOSITIONS AND METHODS AND APPARATUS FOR THE PRODUCTION THEREOF

#### (57) Abstract

There is provided a method of producing protein feedstock compositions from fisheries waste useful as fertilizer, feed supplements for livestock and aquaculture, and nematodicides. Fisheries waste is comminuted and anaerobically digested by Lactobacillus acidophilus at pH 4.5 to 5.0 and at a temperature between 25 °C and 38 °C, screened to yield a substantially liquid phase, and aged for three months. The composition is then screened to 1.5mm and further aged for two months. The composition may be used directly, or may include an oily phase comprising from 0.5 to 5 % by volume of composition, the oily phase being introduced via a self emulsifying master composition of a degummed vegetable oil and nonionic surfactant. Comminution apparatus for said fisheries waste is also described.

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# PROTEIN FEEDSTOCK, COMPOSITIONS AND METHODS AND APPARATUS FOR THE PRODUCTION THEREOF

This invention relates to protein feedstock, compositions and methods and apparatus for the production 5 thereof.

particular This invention has but not exclusive application to fish by-product protein compositions and methods and apparatus for the production thereof, and for illustrative purposes reference will be 10 made to such application. However, it is to be understood that this invention could be used in other applications, by-product protein feedstock, such as other animal compositions and methods and apparatus for the production thereof.

The use of fish processing waste and bycatch as a 15 manufacturing feedstock for fertilizer production acknowledged as a desirable way of putting such wastes to use, due to the well known properties of fish wastes as a source of nitrogen, phosphorous, potassium and 20 minerals required by plants for healthy growth. One form of fertilizer compositions currently produced are the so called fish emulsions, where aqueous slurries of ground fish waste are allowed to decompose, and a liquid suspension harvested therefrom and packaged or distributed in bulk for fertilizer 25 use. Other processes include the production of fish meal fertilizer compositions.

Particular fisheries such as the Australian orange roughy fishery have been the subject of much research into uses for waste, due to the high waste weight ratio of such 30 fisheries. However, a considerable proportion of the orange

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roughy and other fishery wastes cannot be practically utilised in the production of fish meal and fish emulsion The principal reason for this inability to process fish waste lies in the economics of transportation 5 of the wastes from their place of production to the waste Further, waste processing facilities processing facility. are restricted from operating in many areas producing the waste due to the noxious nature of the processes used to produce fertilizer and other products.

The present invention aims to substantially alleviate 10 at least one of the above disadvantages and to provide protein feedstock, compositions and methods and apparatus for the production thereof which will be reliable and efficient in use. Other objects and advantages of this 15 invention will hereinafter become apparent.

With the foregoing and other objects in view, this invention in one aspect resides broadly in a method for production of protein feedstock comprising:-

comminuting fishery waste;

adjusting the pH of said comminuted fishery waste to an 20 acid pH selected to encourage microbial digestion;

microbially digesting said acidified fish waste at a temperature between 25°C and 38°C;

screening said digestate to yield a substantially 25 liquid phase, and

aging said liquid phase.

The fishery waste may comprise one or more of filleting wastes (fish frames), bycatch or waste whole fish, crustacea or the like. The fish waste may be comminuted to an average 30 particle size of less than 25 mm, and is preferably comminuted to an average particle size of from 6mm to 25 mm. The comminution may be done by any suitable means such as by mincing, grinding or chopping, preferably such that the temperature of the waste does not exceed 35°C, and especially about 20°C, either in the bulk matrix or at the shearing point. Most fishery waste streams have a moisture content of from 50 to 99 wt % and the water content of the comminuted fish waste is preferably adjusted to this range.

The pH of the comminuted fishery waste is preferably -10 adjusted to the selected range by means of mineral or organic acid addition. For example, the pH may be adjusted mineral acids such strong addition of the hydrochloric, sulphuric or nitric acids. However, in order reduce the aggressive hydrolysis of fishery waste proteins and polysaccharides, and to reduce the cytotoxicity of the mixture to the digestion microorganisms adapted to low pH conditions, it is preferred that the adjustment in pH be made by the addition of organic acids such as formic 20 acid.

The pH being selected to encourage the growth of the digesting microorganism will at least in part be determined by the range of pH tolerance of the selected microorganism. Preferably, the microorganism is selected from those which grow best in the acid range for reasons described hereinafter. For example, for Lacidophilus digestion, the preferred range of pH is 4.5 to 5.0.

The digestion is preferably performed in a closed digester, and accordingly the digestion is preferably 30 performed by obligate or facultative anaerobes. The

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digestion may be initiated and progressed by the addition of a culture of microorganisms such as those commonly used for the production of cultured milk products. For example, the microorganism may be selected from the yoghurt-forming 5 microorganisms such as Lactobacilli spp. such Lactobacillus acidophilus. Alternatively or additionally, the digestion may be performed by one or more of Bacillus spp. such as B. subtilis or B. licheniformis, Pseudomonas spp. such as P. fluorescens or P. aeruginosa, or Escherichia 10 hermanii. The digestion may be assisted microorganisms native to the fishery waste and amenable to the conditions of processing. Accordingly, the digestion may be done with or without presterilization of the mixture.

The digestion is performed in the specified temperature 15 range, the temperature being maintained by cooling or the digestion accelerated by heating as the case may require. The temperature is preferably in the range of 25°C to 38°C, for the purpose of maintaining high activity of the acidic anaerobes relied upon for the digestion whilst suppressing 20 the activity of thermophilic, often sulphur-reducing (hydrogen sulphide producing), microorganisms.

The digester may be provided with such additives as may be deemed appropriate to encourage the digestion of the fishery waste by the microorganisms. For example, trace 25 minerals, enzymes and cofactors therefor, and vitamins may be added in accordance with the principles and knowledge of the industrial fermentation art. It has been particularly observed by the inventor that the addition of from 0.01 to 0.2 wt % ascorbic acid to the digester has a beneficial effect on the native microflora of fishery wastes.

It has also been surprisingly noted that the acidic, anaerobic flora preferred for the processes of the present invention have a tolerance for certain antimicrobial compounds and compositions which may be advantageously used to reduce competition from less desirable microorganisms.

Accordingly, in a further aspect this invention resides in a method for production of protein feedstock comprising anaerobically digesting an aqueous slurry of fishery waste at a temperature between 25°C and 38°C in the presence of an antimicrobial compound selected to permit survival of acidophilic anaerobes in said slurry.

Preferably, the antimicrobial compound is a compound having a broad spectrum of cytotoxicity to microorganisms such as yeast, mould and bacteria, whilst being well tolerated by the desirable acidophile anaerobes of the present invention. It has determined that sorbic acid and sorbates, used in the range of 0.01 to 0.2 wt % measured as sorbic acid, encourages the activity of the native acidophiles of fishery wastes.

- The digestion is run to conclusion which may be determined by any known objective measure such as the digester temperature tending to ambient temperature without control. In general, the process of digestion is concluded in about four to seven days at 25°C to 38°C respectively.
- The digestate is preferably screened to less than 1.5mm to provide a liquid protein feedstock fraction having some suspended particulates and emulsified components, and a compostable solids fraction. It has been determined that after digestion has concluded, the metabolites of digestion are still reactive. Insofar as these metabolites are

malodorous, although not to the extent of the ammoniacal and sulphidic prior art fermentates, it has been found that to age the digestate at ambient temperatures for approximately two weeks is to substantially reduce the odour associated with the protein feedstock product. Preferably, the proliferation of opportunistic microorganisms during the aging process is reduced by the addition of a preservative such as potassium sorbate.

A typical range of additions and process conditions for digestion of fisheries waste may include pumping comminuted fisheries waste into a closed vessel with addition of a culture of Lactobacillus acidophilus. The Lactobacillus culture may be prepared by growing (for 3 to 7 days at 25 degrees C) 10g of freeze dried bacteria in 20kg molasses and 15 20 litres water, producing a grown culture suitable for treating 1000 litres of fisheries waste.

After allowing the filled vessel to rest for approximately 3 hours, the pH of the mixture will generally require reduction with typically 2 litres of technical nitric acid for each 1000 litres of fisheries waste. Again preferably allowing an approximate three hour rest phase, there may be added addition nutrients for the bacterial culture and to modify the minerals balance of the final product. For example, there may be added cofactors such as ascorbic acid, typically at 1 kg per 1000 litres fishery waste. Other additives may comprise:

20g - 2kg phosphorous acid

200ml - 4Lt phosphoric acid

0 -3Lt technical sulphuric acid

26g - 1kg boric acid

30

5

6 - 200g sodium molybdate

10 - 500g iron as hydrated oxide

10g - 1kg manganese sulphate

10 - 500g copper sulphate

10g - 5kg zinc sulphate

1 - 10kg potassium sulphate

Allowing again a stabilization period of from 1 to 3 hours, the pH may be checked to ensure appropriately acid conditions for the selected digesting microoganism, 10 typically 4.5 to 5.0 for the preferred Lacidophilus.

The digestion as observed is carried out for an appropriate time determined by completion of digestion. avoid premature termination through pH variation outside of the facultative range, the pH may be adjusted up by the 15 addition of a base such as calcium carbonate or down by addition of a mineral or organic acid. After the digestion is complete, the digestate is preferably lowered in pH to the bottom of the facultative range with mineral or organic acid such as formic acid, prior to pumping to a first 20 screening means to remove solids of for example greater than The screened material may then be aged in accordance with the present invention. Typically the digestate may be aged for up to three months, preferably with periodic pump recirculation to encourage homogenous aging. 25 preferably done in a stainless steel tank. Typically the recirculation may be done daily, and the temperature preferably maintained in the range 25°C to 38°C and the pH in the facultative range.

The aged digestate may be further screened, typically 30 down to 1.5mm maximum particle size, and pumped into a

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secondary aging tank of plastic or other inert material. Typically the secondary aging may be for about 2 months. An opportunity exists for the development of fungal or yeast growth. Accordingly, the digested material may be preserved 5 by addition of an inhibitor such as sorbic acid or sorbate salts. Such inhibitors may be added at a rate of 200g per 1000Lt of digestate. In order to further inhibit microbial growth, the pH may be lowered to be strongly acidic, such as to 3.3 by addition of formic acid or the like. Again the 10 temperature is typically maintained in the range 25°C to 38°C.

digestate protein feedstock produced by foregoing methods may find use in any application where a 15 digested animal protein source may be used, such fertilizer compositions, stock feed supplements and aquaculture nutrition.

In a further aspect this invention resides in a fertilizer composition including an aged, liquid protein 20 feedstock produced from fishery waste, said fishery waste being comminuted at a temperature of preferably not more than 35°C, acidified to a facultative pH, microbially digested at a temperature between 25°C and 38°C and screened to yield a substantially liquid phase prior to aging.

The fertilizer compositions of the present invention are preferably in the form of relatively stable emulsions and/or suspensions in a continuous phase comprised in the majority of the protein feedstock of the present invention. The composition may comprise trace elements, antioxidants, 30 preservatives and the like, in stable solution, suspension

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or emulsion in the protein feedstock, in accordance with the additive's phase and solubility. Advantageously, oily phase additives may be emulsified in the composition by the use of a suitable surfactant. Preferably the surfactant is selected from the nonionic surfactants to avoid flocculation or precipitation by charge effects from what is a complex mixture of components the result of digestion of fishery waste. The compositions may be boosted in nutrients if desired by the addition of mineral additives dispersible in one or the other of the phases of the composition.

It has been surprisingly determined that improved performance of the fertilizer compositions is achieved by maintaining an oily phase in the emulsion of from 0.5 to 5 % by volume of composition. Whilst the digested and aged 15 protein feedstock will generally by its nature include a proportion of emulsified fish oil, it may be desirable to increase the oily phase by the addition of, for example, a physiologically compatible oil such as fish oil or vegetable oil. Preferably, the oily addition is a physiologically 20 compatible vegetable oil. In order to ensure proper emulsification of the oil or oily additives compositions, it may be desirable to produce a high-oilcontent stable emulsion in the protein feedstock or other aqueous medium, followed by dilution to specification with 25 the protein feedstock. Preferably, the added oil precharged with oil compatible additives intended for the compositions, and may be premixed with the surfactant necessary to emulsify the oil in the composition.

In the process of developing the embodiments of the 30 compositions of the present invention, it has been determined that particular master compositions comprising the oil phase and emulsifier are particularly advantageous. These compositions are those having reduced oil gum, the oil gum being a consequence of the multiple unsaturation in the fatty acid chains constituting the triglyceride oil. It has been surprisingly determined that a compatible surfactant when mixed with a vegetable oil containing gum will effect the precipitation of the gum from the oil.

Accordingly, in a further aspect this invention resides 10, broadly in a method for producing oil master compositions for use in oil-in-water emulsion fertilizer compositions and comprising:-

treating a vegetable oil with surfactant sufficient to emulsify said oil in an aqueous phase;

removing precipitated gum from said oil, and adding an antioxidant to said treated oil.

The vegetable oil is preferably a mono or polyunsaturated vegetable oil. For example, the vegetable oil may be selected from food or technical grade vegetable oils such as canola, safflower, mustard seed, or peanut oils.

The surfactant is preferably a nonionic surfactant miscible with the vegetable oil or at least soluble therein to the extent necessary to provided a composition with the oil which is emulsifiable in an aqueous phase. For example, the surfactant may be selected from the vegetable oil-soluble nonionic surfactants such as alkoxylated hydroxy-functional triglyceride oils such as castor oil.

The antioxidant may be selected from any of the known 30 antioxidants for unsaturated fats, and is preferably

selected from antioxidants approved for food use. It is particularly preferred to use antioxidants having the potential for uptake by plants as cofactors such as free radical scavenging vitamins or derivatives thereof such as  $dl-\alpha$ -tocopherol acetate.

The master composition may include other oil soluble additives such as other vitamins.

An unexpected result of the application of the vegetable oil adjuvant-containing digestate compositions of the present application has been a marked reduction in aquatic plant biomass. This is in contrast to the usual effect of soluble nutrients being leached into or applied to water courses, resulting in plant proliferation including algal blooms.

Application of the present compositions to a body of water results in the emulsion breaking and a degradable oil film being formed on the water surface. This film appears to prevent the plant from accessing some essential particular despite the stimulant effect of the nutrients dissolved from the composition. The concentrated supply of nutrients in the water appears to overfeed plants such as algae, water hyacinth and salvinia while the plant is getting insufficient usable light and oxygen. Microscopic examination appears to show that the cell walls of the plant break down or rupture.

The digestate compositions of the present invention have also proved to be unexpectedly useful in aquaculture, particularly in fish farming, as food supplement. Typically, the compositions may be utilized at a delivery of a supplement at a delivery and the supplement are of 8 ml per day per kilogram live weight of fish at

liveweight loadings aquaculture typical of Alternatively, the nutrient loading may be maintained at 1 part by volume of composition per 100 to 400 litres of pond water.

As a protein supplement in stock feeds, the digestate compositions may be utilized in lieu of or in addition to the kelp supplements commonly fed to stock to prevent or treat selenium and iodine deficiencies, in horses, cattle and other grazing animals. It has been determined that the 10 deficiencies addressed by kelp supplements are also at least in part addressed by the present compositions. Typically the compositions may be utilized at levels of 15 to 20 ml per beast per day for large animals, with less being required for smaller beasts such as pigs, which may thrive 15 on about 10 ml per day.

The nutritive advantages of the use of compositions in accordance with the present invention may be achieved by direct administration to the beast or addition to drinking water, wherein the compositions are wholly dispersible. 20 has been found that the compositions are advantageously administered for alternate weeks of seven days.

In the production of protein feedstock from fishery waste, it is preferred that the comminution of the waste prior to digestion not result in local of bulk heating above 25 35°C. If the temperature is raised above 35°C, the fishery waste tends to cook, affecting the quality of the final Apparatus for comminuting fishery waste by product. mincing tend to heat the waste quite grinding or considerably.

Accordingly, in a further aspect this invention resides 30

in comminuter apparatus including:-

a housing having an inlet opening including a shearing edge;

a shearing blade rotatable in said housing having a leading edge extending outward from a hub portion of the blade having an outer edge portion curving in the direction of rotation and adapted to cooperate with said shearing edge, and

a plurality of mincing blades mounted for co-rotation 10 with said shearing blade.

The housing may include an internal space of any shape representing a solid of rotation, although it is preferred that the space be substantially cylindrical, the cylindrical surface being defined by an inner housing wall. By this means an outlet may be provided in the form of a perforate portion of the inner housing wall in communication with a space between the inner housing wall and an outer housing wall.

The inlet may comprise an aperture in an end to the housing defined in part by the shearing edge disposed along a chord to a substantially circular end of the housing, the edge comprising the corresponding shearing edge adapted to cooperate with the shearing blade. For example, the shearing edge may be disposed substantially radially of said housing end.

The shearing blade may extend across a selected span of the inlet. However, it is preferred that the shearing blade extend such that it sweeps substantially all of the inlet and that the shearing blade sweep the length of the 30 corresponding edge of the inlet.

The shearing blade edge may curve in a substantially epicycloid shape in the direction of rotation from the hub of the blade. Alternatively, the curvature may have a smooth transition from curving backwards relative to the direction of rotation from the hub portion, preferably for 30-50 % of the blade radius, through a neutral point to an outer tip swept forwards in the direction of rotation.

By this means, material introduced in the region of the hub through the inlet aperture tends to be pushed outward to 10. a region of the blade edge having sufficient linear speed to effectively shear the material. Material entering at the outer region of the shearing blade's sweep would, if the blade were straight, be impelled radially outward. However, the forward curve of the blade tends to at least partially counter this tendency thus promoting proper shearing of the material.

For processing fishery waste, the blade may be set such that a specified clearance is maintained between the blade edge and the shearing edge. For example, experimentation 20 has established that for prawn processing waste, a clearance of about 6 mm produces an particle size spread of the comminuted product suitable for the production of the protein feedstocks of the present invention.

The blade may be one of a plurality of such blades

25 distributed evenly about the axis of rotation of a rotor

mounted within the housing. For example, the apparatus may

be provided with four such blades although it is envisaged

that an odd number or other number of blades may be

selected. Preferably, the apparatus is provided with four

30 such blades mounted in orthogonal sets of two blades each,

with adjacent blades axially staggered such that a clearance of about 6 mm is maintained between the shearing planes of the respective sets. It has been found that this configuration promotes an advantageous distribution of particle size of selected fishery wastes such as prawn processing wastes.

The mashing blades may, for processing the majority of fishery wastes, comprise a plurality of mashing blades which are preferably mounted on a common rotor with the shearing blade or blades. Preferably, the mashing blades are mounted in sets with the sets distributed about the rotary axis and extending from the shearing blade or blades to the end of the housing remote from the inlet.

The rotor may be driven by any suitable means and is preferably driven by an electric motor.

This invention further relates to antinematodal soil treatment methods and compositions therefor derived from the foregoing compositions.

further embodiments of the invention These further application not exclusive 20 particular but antinematodal soil treatment methods for commercial field crops, and compositions suitable for use in these methods, and for illustrative purposes reference will be made to such However, it is to be understood that these application. 25 embodiments of the invention could be used in other applications, such as commercial or domestic horticultural applications.

Nematodes are a serious soil pest of commercial crops.

The present invention aims to substantially alleviate 30 at least one of the above disadvantages and to provide

antinematodal soil treatment methods and compositions therefor will be reliable and efficient in use. Other objects and advantages of this invention will hereinafter become apparent.

With the foregoing and other objects in view, this invention in one aspect resides broadly in an antinematodal soil treatment method comprising applying to the soil a nematodicidally effective amount of a protein composition produced by:

10 comminuting fishery waste;

adjusting the pH of said comminuted fishery waste to an acid pH selected to be facultative for digestion microbes;

microbially digesting said acidified fish waste at a temperature between 25°C and 38°C;

15 screening said digestate to yield a substantially liquid phase, and

aging said liquid phase.

The proteinaceous material may be used directly or distributed in irrigation water. Preferably, the 20 proteinaceous material is applied at a rate of at least 60 L/ha to provide a nematodicidal effect.

In experimentation with application rates it has been surprisingly been determined that a synergism arises when the proteinaceous material is compounded as an emulsion with an emulsifiable oil, in combination with an active culture of a microorganism. The active culture may comprise the dormant culture present in the aged protein product itself or may be added to the emulsifiable composition of oil phase and protein product.

30 In either case it is preferred that the composition

5 emulsifiable oil/protein/bacteria mixtures.

include biologically active <u>Lactobacillus acidophilus</u> at the time of application to the soil. It has been found that effective protein product application rates down to below 40 L/ha are nematodicidally effective using the aforementioned

Advantageously, oily phase additives may be emulsified in the composition by the use of a suitable surfactant. Preferably the surfactant is selected from the nonionic surfactants to avoid flocculation or precipitation by charge effects from what is a complex mixture of components the result of digestion of fishery waste.

It has been surprisingly determined that improved performance of the nematodicidal compositions is achieved by maintaining an oily phase in the emulsion of from 0.5 to 5 % 15 by volume of composition. Whilst the digested and aged protein feedstock will generally by its nature include a proportion of emulsified fish oil, it may be desirable to increase the oily phase by the addition of, for example, a physiologically compatible oil such as fish oil or vegetable 20 oil. Preferably, the oily addition is a physiologically compatible vegetable oil. In order to ensure proper emulsification of the oil or oily additives compositions, it may be desirable to produce a high-oilcontent stable emulsion in the protein feedstock or other 25 aqueous medium, followed by dilution to specification with the protein feedstock. Preferably, the added oil is precharged with oil compatible additives intended for the compositions, and may be premixed with the surfactant necessary to emulsify the oil in the composition.

30 In the process of developing the embodiments of the

compositions of the present invention, it has been determined that the aforedescribed compositions comprising the reduced gum, oil phase and emulsifier are particularly advantageous.

The method of use of the aforementioned preferred compositions may comprise irrigation spraying or direct application to the ground, application of the compositions directly followed by watering in or any other method capable of delivering the composition to the soil horizon containing the nematode infestation. The composition is used at a protein feedstock loading of at least 30 L/ha and preferably at least 40 L/ha. Preferably the mixture is applied by entrainment with irrigation water applied directly to the soil. Protein feedstock used directly is used at least 60 L/ha.

Preferably, the soil is treated at least twice to substantially eliminate nematode infestation. For example, the soil may be rested after a first treatment prior tilling and/or fertilizer application ready for planting, followed 20 by a second treatment, resting then, planting.

In order that this invention may be more readily understood and put into practical effect, reference will now be made to the following examples and accompanying drawings which illustrate a preferred embodiment of one aspect of the invention and wherein:

FIG. 1 is a side view of a shearing blade assembly for comminuter apparatus in accordance with the present invention;

FIG. 2 is side view of the assembly of FIG. 1, and FIG. 3 is a section through comminuter apparatus in

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accordance with the present invention.

In the Figures, there is illustrated comminuter apparatus 10 including an outer substantially cylindrical housing 11 closed at one end by an end plate 12. Mounted within the outer housing 11 is a cylindrical inner housing 13 defining a rotor chamber 14. An annular space 15 between the outer 11 and inner 13 housings communicates with an outlet (not shown) from the apparatus 10.

Closing the end of the inner 13 and outer 11 housings
10 is a closure assembly 16 secured by a bolting ring 17 to a
corresponding bolting flange 20 formed on the periphery of
the outer housing 11. The closure assembly 16 incorporates
a substantially semicircular-section inlet chute 21 bounded
by a curved wall portion 22 abutting a portion of the inner
15 housing 13 and a flat wall portion 23 disposed diametrically
across the end of the inner housing 13.

The flat wall portion 23 is provided with a fixed shearing edge 24 extending into the inner housing 13 and being disposed substantially along a radius of the inner 20 housing 13. The portion of the closure assembly 16 not occupied by the inlet chute 21 is closed by closure portion 25.

Mounted for rotation within the inner housing 13 is a rotor assembly 26 comprising a driven shaft 27 penetrating 25 the end plate 12 and having mounted thereon a plurality of mincing blade assemblies 30 arranged in alternating orthogonal pairs. Towards the end closure assembly 16, the shaft 27 mounts two pairs of shearing blades 31 arranged orthogonally on the shaft 27 and secured by a nut 32. The 30 closest pair of shearing blades 31 to the shearing edge 24

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is mounted such that a clearance 33 of about 6 mm is maintained therebetween.

The shearing blade edges 34 extend outward from the region of the shaft 27 to tips 35, with the outer halves of the blade edges 34 being curved in the direction of rotation of the blades 31, and the inner halves being curved from the region of the shaft 27 away from direction of rotation, the two halves describing a smooth curve meeting at a transition point 36. The outer halves are swept forwards from the transition point 36 by approximately 17.5° of arc, whereas the inner halves are swept back from the shaft 27 to the transition point 36 by approximately 45° of arc.

In use, waste material such as fisheries waste may be manually sorted to remove obvious rubbish and may further be sreened by a metal detector to provide automatic shut down of plant before damage is occasioned. The fishery waste may be optionally blown with air to remove loose scales. The waste may be crushed prior to passing to the mincer. The raw or pretreated waste is fed to the apparatus 10 through the chute 21 whereupon it is sheared by the shearing blades 31. The clearance 33 provides for control of the particle size of the sheared product whilst the shearing action provides that the sheared product presents an appropriate shaped particle to the mincing blades.

25 Effective shearing is maintained by the inner halves of the shearing blade edges 34 urging the material to the neutral point 36 of the blade edges 34 whilst the outer halves act to restrain the centrifugal acceleration of the material to ensure that the majority passes effectively to 30 the mincing blades 30. The mincing blades 30 then further

comminute the material to the desired particle size before discharging the processed meal to the annular space 15 and thence to an outlet via apertures 37 provided through the inner housing 13.

The use of the shearing action reduces the initial heat generation common to the prior art apparatus whilst the mincing stage presents a product of physical form consistent with that produced by the prior art apparatus.

#### EXAMPLE 1 - Protein feedstock

A culture aliquot of Lactobacillus acidophilus was 10 grown by the addition of 20 kg molasses to 20 Lt water to form a growth medium, the temperature of which was adjusted to 25°C prior to the addition of a starter culture of the bacteria or 10g of freeze dried bacteria. Into a 4000 litre 15 digester was placed fisheries waste processed using the apparatus of FIGS 1 to 3 at 20°C, with progressive addition of 4 aliquots of the grown bacterial culture. After 3 hours stabilization the waste was acidified with 8 Lt technical grade nitric acid, and after a further 3 hours stabilization 20 4 aliquots of the following additive mixture were slowly added:

lkg ascorbic acid 2kg phosphorous acid 4Lt phosphoric acid 25 3Lt technical sulphuric acid 26g boric acid 6g sodium molybdate 40g iron as hydrated oxide 45g manganese sulphate 30 35g copper sulphate

40g zinc sulphate

lkg potassium sulphate

After a one hour stabilization period the pH was checked and adjusted to the range 4.5 to 5.0 by addition of 5 calcium carbonate (1.0 micron) or formic acid as required from batch to batch. The digester was then brought up to a temperature of 30°C to initiate active growth by the bacteria. The digester was run for 7 days with the temperature being maintained in the region of 30°C and 10 between 25°C and 38°C. During the digestion, the pH tended to drop and the pH was maintained in the range of 4.5 to 5.0 by calcium carbonate additions.

At the end of the seven day digestion, the digestate was pumped to a vibratory screen to reduce the maximum 15 particle size to 3mm and the pH was reduced to 4.5 by the addition of formic acid. The screened digestate was pumped to a second stainless steel tank, maintained at pH 4.5 to 5.0 and temperature 25 to 38°C and stirred daily by recirculation for 3 months.

The aged digestate was then further screened by 20 vibratory apparatus to reduce the maximum particle size to 1.5 mm before being pumped to a plastic tank and further aged at between 25°C to 38°C for two months with recirculation. The pH was dropped to 3.3 by the addition of 25 formic inactivate the bacteria and other acid to microorganisms, the preservation of the aged digestate being further promoted by the addition of 200g/1000Lt sorbic acid as potassium sorbate.

The solids from the two screenings were combined to 30 produce a fishmeal filtrate for composting.

#### EXAMPLE 2 - OIL ADJUVANT

To 1000 litres of canola cil was added 100 litres of nonionic surfactant (Teric 380, ICI) being an ethoxylated castor oil soluble in canola cil. After mixing, the mixture 5 was transferred to a funnel tank and allowed to stand for 3 days at ambient temperature. At the end of 3 days the vegetable cil gum had separated from the vegetable cil/surfactant solution which was separated from the gum which was then disposed of. To the gum free cil/surfactant solution was added 1 kilogram of dl-α-tocopheryl acetate (vitamin E acetate) and 1.5 kilograms of a composition of vitamin a palmitate in peanut cil containing 1.0 million international units per gram.

#### EXAMPLE 3 - FERTILIZER COMPOSITION 1

- To 3.6 kilograms of boric acid was added 1.5 kilograms of monopotassium phosphate, 112 grams of sodium molybdate, 12 kilograms of potassium sulphate, 5.5 kilograms of magnesium sulphate, 1 kilogram of ascorbic acid and 500 milliliters of surfactant (Teric 9A6).
- 20 500 litres of the protein feedstock of example 1 was added to a paddle mixer and to which the foregoing additives were added with thorough mixing. 15 litres of the oil adjuvant of example 2 was added and the mixture made up to 1000 litres with further protein feedstock of example 1.

## 25 EXAMPLE 4 - FERTILIZER COMPOSITION 2

To 72 kilograms of urea was added 186 kilograms of ammonium sulphate, 94 kilograms of mono ammonium phosphate, 85 kilograms of potassium sulphate, 92 kilograms of potassium nitrate, 3.6 kilograms of boric acid, 112 grams of 30 sodium molybdate, 2 kilograms of zinc sulphate (as

heptahydrat ) and 5.5 kilograms of magnesium sulphate. These ingredients were added to 500 litres of the protein feedstock of example 1 with vigorous stirring in a paddle stirring reactor. To the mixture was added 20 litres of the oil adjuvant of example 2, the mixture mixed to homogeneity to the eye and made up to 1000 litres with further liquid protein feedstock of example 1.

#### EXAMPLE 5 - FERTILIZER COMPOSITION 3

To 1000 kg of cow manure was added 200 kg minced orange 10 peel, 40 litres of phosphoric acid, 20 to 40 kg fish bone meal, 40 kg superphosphate (TRIFOS, Incitec), 12 kg potassium sulphate, 5.3 kg boric acid, 100 g sodium molybdate, 1.1 kg zinc sulphate monohydrate and 10-20 litres of the liquid protein feedstock of Example 1. The moisture content of the mixture was adjusted to be in the region of 15-35% by weight by the addition of further liquid protein feedstock to aid processing.

The mixture was mixed to be substantially homogeneous and then transferred to a mixing vacuum oven, where the 20 temperature was increased to between 45-50°C under vacuum to partially sterilize the composition and reduce the moisture content to a packagable level.

#### EXAMPLE 6 - NEMATODICIDAL COMPOSITION

A l hectare plot of farmland having a commercially significant nematode infestation was identified. To 40 parts by volume of the protein feedstock of example 1 was added 1 part by volume of the oil adjuvant of example 2, with mixing until an emulsified composition was formed. To the mixture was added an active culture of <u>Lactobacillus</u> 30 acidophilus with mixing. The final mixture was then

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turbulently dispersed into an irrigation stream and applied to the soil at a net rate of 40 L/ha of protein feedstock.

After 4 days, the soil was watered to a tillable state and organic fertilizer granules, in this case the fish waste residue of the process of Example 1 processed to granules, were applied to the plot at the rate of 1000 kg per hectare. The plot was then tilled by rotary hoe to 150-200 mm prior to a second treatment with the nematodicidal mixture at 40 L/ha of protein feedstock. After a further 4 day rest, the plot was watered and planted. Periodic assays determined that nematode loadings were sub-infestational and did not increase over the month after planting.

It will of course be realised that while the above has been given by way of illustrative example of this invention, all such and other modifications and variations thereto as would be apparent to persons skilled in the art are deemed to fall within the broad scope and ambit of this invention as defined in the claims appended hereto.

#### CLAIMS

1. A method for production of protein feedstock comprising:-

comminuting fishery waste;

adjusting the pH of said comminuted fishery waste to an acid pH selected to encourage microbial digestion;

microbially digesting said acidified fish waste at a temperature between 25°C and 38°C;

screening said digestate to yield a substantially liquid phase, and

aging said liquid phase.

- 2. A method according to Claim 1, wherein said fishery waste is selected from one or more of filleting wastes (fish frames), bycatch or waste whole fish, crustacea or the like.
- 3. A method according to Claim 2, wherein said fishery waste is comminuted to an average particle size of from 6mm to 25 mm.
- 4. A method according to any one of the preceding Claims wherein said comminution is done such that the temperature of the waste does not exceed  $35^{\circ}$ C.
- 5. A method according to any one of the preceding Claims wherein the moisture content of said comminuted fisheries waste is selected or adjusted to within the range 50 to 99 wt %.
- A method according to any one of the preceding Claims,

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wherein said digesting microorganism is selected from acidophilic bacteria being obligate or facultative anaerobes.

- 7. A method according to Claim 6, wherein said bacteria is Lactobacillus acidophilus.
- 8. A method according to Claim 7, wherein the pH of the digestion is maintained in the range of 4.5 to 5.0.
- 9. A method according to Claim 8 wherein said digestion is done in the presence of from 0.01 to 0.2 wt % ascorbic acid.
- 10. A method according to any one of Claims 6 to 9 wherein said digestion is done in the presence of an antimicrobial compound selected to permit survival of acidophilic anaerobes in said digestion.
- 11. A method according to Claim 10, wherein said antimicrobial compound is selected from sorbic acid and sorbates, used in the range of 0.01 to 0.2 wt % measured as sorbic acid.
- 12. A method according to any one of the preceding Claims wherein after the digestion is complete the digestate is screened through first screening means to remove solids of greater than 3mm.
- 13. A method according to any one of the preceding Claims, wherein the digestate is ages for up to three months.

- 14. A method according to Claim 13, wherein said aging is accompanied by periodic pump recirculation to encourage homogenous aging.
- 15. A method according to Claim 14, wherein during aging the temperature is maintained in the range 25°C to 38°C and the pH in the facultative range.
- 16. A method according to Claim 15, wherein said aged digestate is screened to 1.5mm maximum particle size.
- 17. A method according to Claim 16, wherein said screened digestate is pumped into a secondary aging tank of plastic or other inert material and given a secondary aging of about two months maintained in the range 25°C to 38°C.
- 18. A method according to Claim 17, wherein said secondarily aged digestate is preserved by lowering the pH and addition of an inhibitor selected from sorbic acid and sorbate salts.
- 19. A digestate protein feedstock produced by a method as defined in any one of the preceding Claims.
- 20. A fertilizer composition including the digestate protein feedstock of Claim 19 and an oily phase emulsified therewith, said oily phase comprising from 0.5 to 5 % by volume of composition.
- 21. A fertilizer composition according to Claim 20, wherein

said oily phase comprises a physiologically compatible oil selected from fish oil or vegetable oil.

- 22. A fertilizer composition according to Claim 21, wherein said only phase is provided by a master compositions comprising the oil phase and emulsifier.
- 23. A method for producing oil master compositions for use in oil-in-water emulsion fertilizer compositions and comprising:-

treating a vegetable oil with surfactant sufficient to emulsify said oil in an aqueous phase;

removing precipitated gum from said oil, and adding an antioxidant to said treated oil.

- 24. A method according to Claim 23, wherein said surfactant is a nonionic surfactant miscible with the vegetable oil or at least soluble therein to the extent necessary to provided a composition with the oil which is emulsifiable in an aqueous phase.
- 25. A method according to Claim 24, wherein said antioxidant  $dl-\alpha$ -tocopherol acetate.
- 26. Comminuter apparatus including:-
- a housing having an inlet opening including a shearing edge;
- a shearing blade rotatable in said housing having a leading edge extending outward from a hub portion of the blade having an outer edge portion curving in the direction

of rotation and adapted to cooperate with said shearing edge, and

a plurality of mincing blades mounted for co-rotation with said shearing blade.

27. An antinematodal soil treatment method comprising applying to the soil a nematodicidally effective amount of a protein composition produced by:

comminuting fishery waste;

adjusting the pH of said comminuted fishery waste to an acid pH selected to be facultative for digestion microbes;

microbially digesting said acidified fish waste at a temperature between 25°C and 38°C;

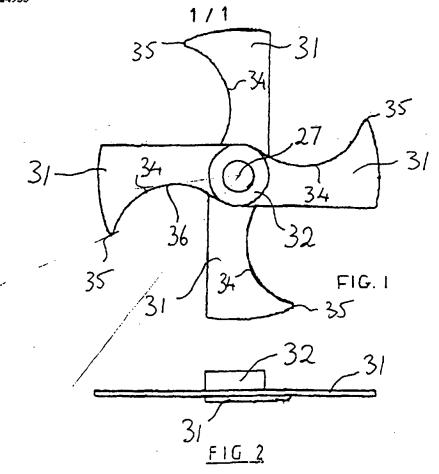
screening said digestate to yield a substantially liquid phase, and

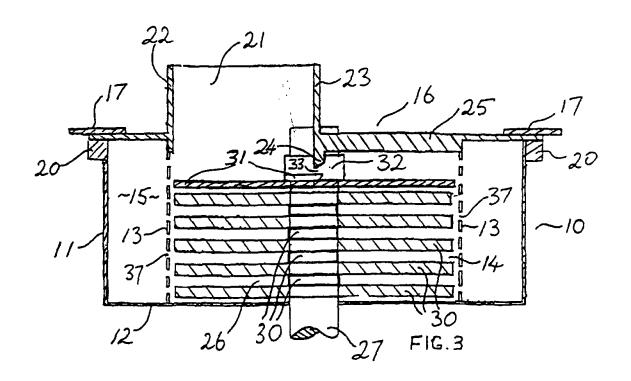
aging said liquid phase.

- 28. A method according to Claim 27, wherein said protein composition is applied to the soil at a rate of at least 60 L/ha.
- 29. A method according to Claim 27, wherein said protein material is compounded as an emulsion with an emulsifiable oil, in combination with an active culture of a microorganism.
- 30. A method according to Claim 30, wherein said active culture comprises the dormant culture present in the aged protein product.
- 31. A method according to Claim 30, wherein said active

culture comprises Lactobacillus acidophilus.

- 32. A method according to Claim 31, wherein the composition is applied at an effective protein product application rate of about 40 L/ha.
- 33. A method according to any one of Claims 29 to 32, wherein said oily phase additive is emulsified in the composition by the use of a nonionic surfactants.
- 34. A method according to Claim 33, wherein said oily phase comprises from 0.5 to 5 % by volume of composition.





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#### A. CLASSIFICATION OF SUBJECT MATTER

Int CI<sup>6</sup>: A23J 1/04; A23K 1/10; C05F 1/00; A23K 1/16; A23J 3/04; C05G 5/00; A01N 63/02; C11B 3/16; A23D 7/04; B02C 18/10, 18/12, 18/18, 18/30; A47J 43/04, 43/07

According to International Patent Classification (IPC) or to both national classification and IPC

#### B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
A23J 1/04, 3/04; A23K 1/10, 1/16; C05F 1/00; C05G 5/00; A01N 63/02; A23D 7/04; C11B 3/16; B02C 18/10, 18/12, 18/18, 18/30; A47J 43/04, 43/07

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched AU:IPC as above

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used) DERWENT:- FISH/CRUSTACEA; PROTEIN; SURF.; ANTIOXID; BLADE/KNIFE/KNIVE; OIL

FSTA:- FISH/CRUSTACEA; PROTEIN

C.	DOCUMENTS CONSIDERED TO BE RELEVANT			
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.		
x	Derwent Abstract Accession No. 85-125191, Class D12, and JP,A, 60-062962 (TAIYO FISHERY KK),11 April 1985.	126710		
	Derwent Abstract Accession No. 88-114995, Class C04 D16, and JP,A, 63-060188 (OSUKAI), 16 March 1988.	1-2, 6-7, 19		
Α	abstract	20-22, 27-34		
A	Derwent Abstract Accession No.85-143930, Class D12 (D23), and JP,A, 60-078548 (ASAHI DENKA KOGYO), 4 May 1985. abstract	1-22		
x	Further documents are listed in the continuation of Box C X See patent family annex			
*Special categories of cited documents:  "A" document defining the general state of the art which is not considered to be of particular relevance earlier document but published on or after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone or which is cited to establish the publication date of another citation or other special reason (as specified)  "O" document referring to an oral disclosure, use, exhibition or other means document published prior to the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art document member of the same patent family				
Date of the actual completion of the international search  29 April 1997  Date of mailing of the international search report  0 7.0 5.9 7				
Name and mailing address of the ISA/AU AUSTRALIAN INDUSTRIAL PROPERTY ORGANISATION PO BOX 200 WODEN ACT 2606 AUSTRALIA Facsimile No.: (06) 285 3929  Authorized officer  Authorized officer  ASHENAFI TESSEMA  Telephone No.: (06) 283 2271				

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C (Continua	tion) DOCUMENTS CONSIDERED TO BE RELEVANT	
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US ,A, 5,393,318 (MITSUOIIZUKA, YAIZU ET AL.), 28 February 1995. col. 1, line 39-col. 2, line 7	1-23, 27-34
Α	US,A, 3,561,973 (MAX RUTMAN), 9 February 1971 whole document	1-25
×	FR,A, 2,702,774 (CIRAD CENT COOP INT EN RECH AGRONOMIQUE), 23 March 1994, whole document	23-25
x	WO, A, 94/05298 (PHARMOS CORPORATION), 17 March 1994. page 5, lines 1-25; page 9, line 23 - page 10, line 9; page 10, line 29 - page 11, line 10; page 11, lines 15-23	23-25
Y	Derwent Abstract Accession No. 92-054038, Class D23, and JP,A, 04-001296 (NISHIN OIL MILLS KK), 6 January 1992. abstract	23-25
Y	Derwent Abstract Accession No. 95-049052, Class A97 E17 G02, and JP,A, 06-329970 (TOHOKU RIKO KK), 29 November 1994. abstract	23-25
Y	EP,A, 507363 (N.V. VANDEMOORTELE INTERNATIONAL), 7 October 1992. page 2, line 58 - page 3, line 45; examples 1-2	23-25
Y	WO,A, 95/03712 (GOLDENBERG VI), 9 February 1995 whole document	23-25
X,P	GB,A, 2,297,759 (SOCIETE DES PRODUITS NESTLE S. A.), 14 August 1996 page 2, lines 4-10; page 3, lines 6-9; page 4, lines 5-12, 34-36; page 5, lines 1-11; example 4	23-25
A	Derwent Abstract Accession No. 96-236352, Class D23, and JP,A, 08-092586 (MIURA JIMUSHO KK). abstract	23-25
P,A	AU, A., 37859/95 (THE LUBRIZOL CORPORATION), 23 May 1996. whole document	20-25
x	Derwent Abstract Accession No. 92-413437, Class P41, and SU,A, 1,694,216 (MOSMASH RES DES TECHN INST), 30 November 1991 abstract	26
x	Derwent Abstract Accession No. 91-013571, Class P41, and SU,A, 1,544,491 (MOSCOW MEAT DAIRY INST), 23 February 1990. abstract	26
x	AU,A, 41842/93 (BRENTWOOD ENGINEERING PTY LTD), 20 January 1994. Figures 1 and 2; claims 1-6	26

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C (Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.	
US,A, 4,600,160 (R. E. MENGEL), 15 July 1986. Figure 1; claim1	26	
WO,A, 95/09051 (CONSUL T.S. DI ROGGERO GIANMARCO & C. S. N. C), 6 April 1995 whole document	26	
<i>\</i>		
	Citation of document, with indication, where appropriate, of the relevant passages  US,A, 4,600,160 (R. E. MENGEL), 15 July 1986. Figure 1; claim!  WO,A, 95/09051 (CONSUL T.S. DI ROGGERO GIANMARCO & C. S. N. C), 6 April 1995	

International Application No.
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Box 1	Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)
This In reasons	sternational Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following
1.	Claims Nos.: because they relate to subject matter not required to be searched by this Authority, namely:
2.	Claims Nos.:  because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3.	Claims Nos.:  because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a)
Box II	Observations where unity of invention is lacking (Continuation of item 2 of first sheet)
This Int	ternational Searching Authority found multiple inventions in this international application, as follows:
(1) (2) (3)	Claims 1-19, 20-22 and 27-34 - methods of producing protein feedstock & its application, Claims 23-25 - methods of producing oil master compositions, Claim 26 - comminuter apparatus,
	as reasoned on the extra sheet.
1.	As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims
2.	As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3.	As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4.	No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:
Remark	k on Protest The additional search fees were accompanied by the applicant's protest.
	No protest accompanied the payment of additional search fees.

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#### Box II Observation where unity of invention is lacking (Continuation of item 2 of first sheet)

The international application does not comply with the requirements of unity of invention because it does not relate to one invention or to a group of inventions so linked as to form a single general inventive concept. In coming to this conclusion, the International Searching Authority has found that there are three inventions:

- A. (i) Claims 1-19 are directed to methods of producing protein feedstock from fishery waste, and protein feedstock produced by the methods.
  - (ii) Claims 20-22 are directed to fertilizer compositions comprising the protein feedstock produced according to claims 1-19.
  - (iii) Claims 27-34 are directed to antinematodal soil treatment methods comprising applying protein feedstock obtained from fishery waste.
- B. Claims 23-25 are directed to methods of producing oil master compositions for use in oil-in-water emulsion fertilizer compositions comprising treating vegetable oil with surfactant, separating the oil from the gum and adding antioxidant to the treated oil. These claims do not include the protein feedstock produced by the method of claim 1.
- C. Claim 26 is directed to a comminuter apparatus for comminuting any product and not necessarily fishery waste.

The common feature among group A claims which can be considered as a "special technical feature" within the meaning of PCT Rule 13.2 is the "protein feedstock produced from fishery waste". However, there is no such common feature between claim groups A and B, A and C or B and C. No technical relationship within the meaning of the PCT Rule 13 exists amongst these groups of inventions, i.e. these groups of inventions are not so linked as to form a single general inventive concept.

Consequently, the international application does not satisfy the requirements of unity of invention.